

WHAT IS CLAIMED IS:

- 1                   1.       A method for identifying a compound that modulates aging, the  
2   method comprising the steps of:  
3                   (i) contacting the compound with a polypeptide, wherein the polypeptide is  
4   encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid  
5   listed in Tables 1 or 3-7, or a nucleic acid encoding a polypeptide listed in Tables 1 or 3-  
6   7or mammalian homologs and orthologs thereof; and  
7                   (ii) determining the functional effect of the compound upon the  
8   polypeptide.
- 1                   2.       The method of claim 1, wherein the homolog or ortholog is a  
2   human homolog or ortholog.
- 1                   3.       The method of claim 2, wherein the human homolog or ortholog is  
2   a human cellular stress-response gene, a human antimicrobial gene, a human metabolic  
3   gene, a human steroid or lipid-soluble hormone synthesis gene, or a human fatty acid  
4   desaturation gene.
- 1                   4.       The method of claim 2, wherein the human homolog or ortholog is  
2   a cytochrome P450, an estradiol-17- $\beta$ -dehydrogenase, a alcohol/short-chain  
3   dehydrogenase, an esterase, a UDP-glucuronosyltransferase, an aminopeptidase, a  
4   carboxypeptidase, an amino-oxidase, an aminoacylase, an oligopeptide transporter,  
5   metallothionein, a receptor guanylate cyclase, a mitochondrial superoxide dismutase, a  
6   catalase, lysosyme, saposin, vitellogenin, glutathione-S-transferase, heat-shock protein,  
7   heat shock factor, an F-box/cullin/Skp protein, an isocitrate lyase, a malate synthase  
8   ASMTL, insulin, IFG1 or IFG2.
- 1                   5.       The method of claim 1, wherein the polypeptide is encoded by a  
2   nucleic acid that hybridizes under stringent conditions to a nucleic acid listed in Tables 5-  
3   6, or a nucleic acid encoding a polypeptide listed in Tables 5-6, or mammalian homologs  
4   or orthologs thereof.
- 1                   6.       The method of claim 1, wherein the functional effect is determined  
2   *in vitro*.

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1                   7.       The method of claim 6, wherein the functional effect is determined  
2 by measuring enzymatic activity.

1                   8.       The method of claim 6, wherein the functional effect is determined  
2 by measuring ligand, substrate, or cofactor binding to the polypeptide.

1                   9.       The method of claim 6, wherein the functional effect is determined  
2 by measuring interaction between a nucleic acid and the polypeptide.

1                   10.      The method of claim 1, wherein the polypeptide is expressed in a  
2 eukaryotic host or host cell and the polypeptide is contacted with the compound in a  
3 living cell.

1                   11.      The method of claim 10, wherein the host cell is derived from *C.*  
2 *elegans*, mouse, rat, or human.

1                   12.      The method of claim 10, wherein the host is *C. elegans*, mouse, rat,  
2 or human.

1                   13.      The method of claim 10, wherein the functional effect is a  
2 determined by measuring ligand, substrate, or cofactor binding to the polypeptide.

1                   14.      The method of claim 10, wherein the functional effect is  
2 determined by measuring transcriptional activation.

1                   15.      The method of claim 10, wherein the functional effect is  
2 determined by evaluating age-associated parameters.

1                   16.      The method of claim 10, wherein the functional effect is  
2 determined by evaluating expression of an age-associated gene.

1                   17.      The method of claim 15, wherein the age-associated parameter is  
2 lifespan.

1                   18.      The method of claim 1, wherein the modulation is inhibition of  
2 aging.

1                   19.     The method of claim 1, wherein the compound is an antibody, an  
2 antisense molecule, or a small molecule.

1                   20.     The method of claim 18, wherein inhibition of aging occurs by  
2 inhibition of a polypeptide encoded by a nucleic acid that hybridizes under stringent  
3 conditions to a nucleic acid encoding a polypeptide comprising an amino acid sequence  
4 selected from the group consisting of the genes listed in Tables 1 or 3-7 or human  
5 homologs and orthologs thereof.

1                   21.     A method for evaluating a compound for modulation of aging, the  
2 method comprising the steps of:  
3                   (i) contacting the compound with a polypeptide, wherein the polypeptide is  
4 encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid  
5 listed in Tables 1 or 3-7, or a nucleic acid encoding a polypeptide listed in Tables 1 or 3-  
6 7, or mammalian homologs and orthologs thereof  
7                   (ii) determining the functional effect of the compound upon the  
8 polypeptide; and  
9                   (iii) contacting a host or host cell expressing the protein and evaluating an  
10 age-associated parameter of the host or host cell, thereby evaluating a compound for  
11 modulation of aging.

1                   22.     The method of claim 21, wherein the homolog or orthologs is a  
2 human homolog or ortholog.

1                   23.     The method of claim 22, wherein the human homolog or ortholog  
2 is a human cellular stress-response gene, a human antimicrobial gene, a human metabolic  
3 gene, a human steroid or lipid-soluble hormone synthesis gene, or a human fatty acid  
4 desaturation gene.

1                   24.     The method of claim 22, wherein the human homolog or ortholog  
2 is a cytochrome P450, an estradiol-17- $\beta$ -dehydrogenase, a alcohol/short-chain  
3 dehydrogenase, an esterase, a UDP-glucuronosyltransferase, an aminopeptidase, a  
4 carboxypeptidase, an amino-oxidase, an aminoacylase, an oligopeptide transporter,  
5 metallothionein, a receptor guanylate cyclase, a mitochondrial superoxide dismutase, a  
6 catalase, lysosyme, saposin, vitellogenin, glutathione-S-transferase, heat-shock protein,

7 an F-box/cullin/Skp protein, an isocitrate lyase, a malate synthase ASMTL, insulin, IFG1  
8 or IFG2.

1 25. The method of claim 21, wherein the polypeptide is encoded by a  
2 nucleic acid that hybridizes under stringent conditions to a nucleic acid listed in Tables 5-  
3 6, or a nucleic acid encoding a polypeptide listed in Tables 5-6, or mammalian homologs  
4 and orthologs thereof.

1 26. The method of claim 21, wherein the polypeptide is recombinant.

1 27. The method of claim 21, wherein the compound is an antibody, an  
2 antisense molecule, or a small molecule.

1 28. The method of claim 21, wherein the functional effect is a physical  
2 effect.

1 29. The method of claim 21, wherein the functional effect is a chemical  
2 effect.

1 30. The method of claim 21, wherein the functional effect is a  
2 phenotypic effect.

1 31. The method of claim 21, wherein the functional effect is  
2 determined *in vitro*.

1 32. The method of claim 21, wherein the functional effect is  
2 determined in a eukaryotic host organism or host cell.

1 33. The method of claim 21, wherein the age-associated parameter is  
2 lifespan, wherein the age-associated parameter is stress resistance.

1 34. A compound that modulates an aging process, wherein the  
2 compound is identified by the method of claim 1 or 21.

1 35. The compound of claim 34, wherein the compound is an antibody,  
2 an antisense molecule, or a small molecule.

1                   36.     A method of modulating lifespan regulation in a subject, the  
2 method comprising the step of administering to the subject an effective amount of a  
3 compound identified using the method of claim 1 or 21.

1                   37.     The method of claim 36, wherein the subject is an adult.

1                   38.     The method of claim 37, wherein the subject is a non-diabetic, non-  
2 obese adult.

1                   39.     The method of claim 37, wherein the subject is not at risk for or  
2 does not have a premature aging disorder.

1                   40.     The method of claim 37, wherein the subject is a healthy adult

1                   41.     A method of increasing lifespan or treating premature aging in a  
2 subject, the method comprising the step of administering to the subject an effective  
3 amount of a compound identified using the method of claim 1 or 21.

1                   42.     The method of claim 41, wherein the aging process is abnormal.

1                   43.     The method of claim 42, wherein the abnormal aging process is  
2 selected from Werner syndrome, Hutchinson-Guilford disease, Bloom's syndrome,  
3 Cockayne's syndrome, ataxia telangiectasia, and Down's syndrome.

1                   44.     The method of claim 41, wherein the aging process is normal.

1                   45.     The method of claim 41, further comprising the step of evaluating  
2 an age-associated parameter of the subject.

1                   46.     A method of identifying a compound that modulates aging, the  
2 method comprising the steps of:

3                   (i) contacting a test compound to a living or biochemical system that comprising a  
4 *C. elegans* target protein selected from the group consisting of: a protein in Tables 1 or 3-  
5 7; and

6                   (ii) evaluating a property associated with the target protein; and

7                   (iii) evaluating an aging-associated parameter of a *C. elegans* organism contacted  
8 with the test compound.

1                   47.     A *C. elegans* nematode that (1) has a deficiency in at least some  
2 cells for an endogenous activity, the deficiency generated by dsRNA in the cells, and (2)  
3 has an average lifespan of at least 40% greater than an otherwise identical nematode  
4 without the deficiency.

1                   48.     A method of identifying a gene or gene product that modulates  
2 aging, the method comprising the steps of:  
3               (i) providing the nematode of claim 47;  
4               (ii) introducing a heterologous gene that encodes a heterologous polypeptide into  
5 the nematode;  
6               (iii) expressing the heterologous gene in the nematode or a progeny of the  
7 nematode under conditions wherein the heterologous polypeptide is produced; and  
8               (iv) monitoring an age-associated parameter of the nematode or the progeny of the  
9 nematode.

1                   49.     The method of claim 48, further comprising contacting a test  
2 compound to the nematode or the progeny prior to or during the monitoring.

1                   50.     A method of evaluating a plurality of compounds, the method  
2 comprising the steps of:  
3               providing a plurality of compounds;  
4               for each compound of the plurality, evaluating a functional effect of the  
5 respective compound on a polypeptide that is encoded by a nucleic acid that hybridizes  
6 under stringent conditions to a nucleic acid listed in Tables 1 or 3-7, or a nucleic acid  
7 encoding a polypeptide listed in Tables 1 or 3-7 or mammalian homologs and orthologs  
8 thereof; and

9               if the compound has a functional effect as determined by a criterion,  
10 contacting the compound to a cell or organism, and evaluating an-age related parameter  
11 of the cell or organism.

1                   51.     The method of claim 50 wherein the criterion is a preselected  
2 value.

1                   52.     The method of claim 50, wherein the criterion is a preselected  
2 statistical significance.

1                   53.     The method of claim 50, wherein the plurality of compounds  
2 comprises a library of structurally related chemical compounds.

1                   54.     A method of altering lifespan regulation in a cell or organism, the  
2 method comprising: increasing expression of at least two class 1 genes in the cell or  
3 organism.

1                   55.     The method of claim 54 wherein the step of increasing comprises  
2 introducing one or more heterologous nucleic acid that encode the at least two class 1  
3 genes.

1                   56.     The method of claim 54, wherein the step of introducing comprises  
2 introducing a single nucleic acid that comprises coding sequences for the at least two  
3 class 1 genes.

Table 1

Clones from Chromosome I screen that suppress *glp-1* longevity, but have minimal effect on wildtype (N2) lifespan

RNAi Clone	<i>glp-1</i> Lifespan			Wild-type			Gene Function (based on homology)
	(days)	% of control	Lifespan (Days)	(N2)	% of control		
vector	24.6	-	19.8		-		empty vector
<i>daf-12</i>	17.7	72	17.2		87		<i>daf-12</i>
<i>daf-16</i>	14.7	60	15.9		80		<i>daf-16</i>
ZK265.1	17.6	72	19.8		100		human G9A protein-like
F49B2.5 (kin-22)	16.1	65	19.5		98		kinase
Y63D3A.3	17.6	72	19.1		96		Unknown, has F box domain
ZC328.3	14.8	60	19		96		Unknown, has TM domain
Y18D10A.10	17.6	72	18.2		92		member of C-type lectin family
K12C11.4	18.25	74	17.8		90		S/T protein kinase
F35E2.3	17	69	17.3		87		Unknown
C06A5.7a (tmd-1)	18.9	77	17.2		87		cytoskeletal regulator
T22A3.5	19	77	17		86		dsRNA binding
F31C3.6	15.5	63	15.7		79		Unknown, has TM domain
B0025.1a (vps-34)	14.9	61	15.5		78		PI3 Kinase, vesicular transport

H R K Y I E L D C T X



Table 2

RNAi clone	Gene Function (Based on homology)	daf-2 lifespan (days)	% of control
vector	empty vector	34.3	-
daf-12	nuclear hormone receptor	33.9	99
daf-16	forkhead transcription factor	14.5	42
ZK265.1	human G9A protein like	26.1	76
F49B2.5 (kin-22)	kinase	33.4	97
Y63D3A.3	unknown, has F box domain	30.3	88
ZC328.3	unknown, has TM domain	37.9	110
Y18D10A.10	member of C-type lectin family	29.2	85
K12C11.4	S/T protein kinase	35.1	102
F35E2.3	unknown	32.3	94
C06A5.7a (tmd-1)	cytoskeletal regulator	34.4	100
T22A3.5	dsRNA binding	41.5	121
F31C3.6	unknown, has TM domain	33.1	97
B0025.1a (vsp-34)	PI3 kinase, vesicular transport	40.8	119

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CF512 (fer-15; fem-1) on RNAi bacteria, whole life, 25C (Sept. 2001)

Note: This strain has no self-progeny when treated at 25C at L3 due to the fer-15 and fem-1 mutations (prevent spermatid formation). RNAi bacteria are from J. Ahlinger's Chr. I, II, and X libraries; both Group 1 (up in daf-2 mutants) and Group 2 genes (down in daf-2 mutants) were tested (see list).

CF512 eggs were placed on RNAi lawns, raised at 25C, and transferred every 4-7 days to fresh RNAi plates.

Shaded cells are not significantly different from control vector bacteria lifespan (p-value>0.05).

		mean	std err	p-value (logrank)	% vector	% avg. V
C54G4.6	ASMTL/MAF	16.92	0.758	<0.0001	129.1603	110.3718
Daf-2		17.1	0.834	0.0002	130.5343	111.5459
C04F6.1	vit-5	15.92	0.626	0.0008	121.5267	103.8486
Y54G11A.6	ctl-1	16.3	0.748	0.0008	124.4274	106.3274
ZK1320.2	unknown	14.63	0.459	0.0017	111.6793	95.43378
ZK270.2	unknown	10.54	0.536	0.0018	80.45801	68.75407
F08B1.1	vhp-1	15.5	0.644	0.0027	118.3206	101.1089
R03E9.1	mdl-1, MAD tf	15.8	0.522	0.0027	120.6106	103.0658
T10B9.1	Cyt. P450	10.9	0.443	0.0035	83.20610	71.10241
K07A1.7	unknown	15.12	0.617	0.0062	115.4198	98.63013
ZK355.E	unknown	15.2	0.585	0.0083	116.0305	99.15198
H22K11.1	asp-3	15	0.708	0.0135	114.5038	97.84735
C17G1.4	mucin	11.38	0.477	0.0181	86.87022	74.23352
K11D2.2	ASAH acid ceramidase	14.86	1.024	0.1281	113.4351	96.93411
F32A5.5	aquaporin	14.37	0.63	0.1548	109.6946	93.73776
T25C12.3	C-type lectin	14.34	1.02	0.1594	109.4656	93.54207
F35H12.2		14	0.562	0.1946	106.8702	91.32420
C18B2.3		12.22	0.495	0.211	93.28244	79.71298
K10B3.8	gpd-2	14.05	0.679	0.2351	107.2519	91.65035

Table 3, cont'd

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**CFS12 (fer-15; fem-1) on RNAi bacteria, whole life, 25C (Sept. 2001)**

Note: This strain has no self-progeny when treated at 25C at L3 due to the fer-15 and fem-1 mutations (prevent spermatid formation). RNAi bacteria are from J. Ahringer's Chr. I, II, and X libraries; both Group 1 (up in daf-2 mutants) and Group 2 genes (down in daf-2 mutants) were tested (see list).

CFS12 eggs were placed on RNAi lawns, raised at 25C, and transferred every 4-7 days to fresh RNAi plates.

Shaded cells are not significantly different from control vector bacteria lifespan (p-value>0.05).

	mean	std err	p-value (logrank)	% vector	% avg. V
F48D6.4	14.07	1.06	0.2475	107.4045	91.78082
F59D8.2	12.5	0.541	0.4161	95.41984	81.53946
T25C12.2	13.72	0.874	0.4515	104.7328	89.49771
Y54G11A.5b	13.53	0.808	0.4688	103.2824	88.25831
C17G10.5	13.53	0.846	0.5635	103.2824	88.25831
F56G4.3	14.84	0.684	0.565	113.2824	96.80365
C44E4.2	13.39	0.79	0.6158	102.2137	87.34507
C55B7.4	13.5	0.885	0.6251	103.0534	88.06262
F21F3.3	12.73	0.501	0.6391	97.17557	83.03979
H16D19.1	13.22	0.778	0.7117	100.9160	86.23613
C40H5.1	12.68	0.736	0.7176	96.79389	82.71363
T01A4.1	13.19	0.734	0.7199	100.6870	86.04044
Daf-16	13	0.519	0.853	99.23664	84.80104
T07D10.4	12.96	1.03	0.8954	98.93129	84.54011
vector	13.1	0.532			85.45335

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Table 3, cont'd

## CF512 (fer-15; fem-1) on RNAi bacteria, whole life, 25C (Dec. 2001)

No self-progeny; RNAi from all chromosomes; experiment as described above.

	mean	std err	p-value	% vector	% avg. V
B0554.1	12.67	0.417	<0.0001	86.78082	82.64840
B0554.6	12.43	0.4	<0.0001	85.13698	81.08284
C04F6.1	12.52	0.372	<0.0001	85.75342	81.66992
C17G1.4	12.21	0.41	<0.0001	83.63013	79.64774
C32H11.10	12.92	0.493	<0.0001	88.49315	84.27919
C32H11.12	12.1	0.38	<0.0001	82.87671	78.93020
C55B7.4	13.04	0.431	<0.0001	89.31506	85.06196
Daf-16	8.34	0.288	<0.0001	57.12328	54.40313
Daf-2	16.017	0.235	<0.0001	109.7054	104.4814
F28D1.3	12.28	0.442	<0.0001	84.10958	80.10437
F28D1.5	12.29	0.463	<0.0001	84.17808	80.16960
F55G11.5	12.1	0.356	<0.0001	82.87671	78.93020
K04E7.2	10.6	0.315	<0.0001	72.60273	69.14546
K07C6.4	12.13	0.411	<0.0001	83.08219	79.12589
T10B9.1	10.77	0.309	<0.0001	73.76712	70.25440
W08D2.4	12.13	0.241	<0.0001	83.08219	79.12589
ZK384.4	12.6	0.401	<0.0001	86.30136	82.19178
ZK1251.2	16.4	0.19	0.0001	112.3287	106.9797
C46F4.2	13.33	0.386	0.0002	91.30136	86.95368
K12G11.3	12.58	0.396	0.0002	86.16438	82.06131
W06D12.3	13.69	0.429	0.0005	93.76712	89.30202

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Table 3, cont'd

	mean	std err	p-value	% vector	% avg. V
T13F2.1	13.12	0.369	0.0008	89.86301	85.58382
C32F10.4	13.59	0.381	0.0014	93.08219	88.64970
Y38H6C.5	13.25	0.37	0.0036	90.75342	86.43183
B0213.15	14.35	0.365	0.0129	98.28767	93.60730
F11A5.12	13.93	0.407	0.0147	95.41095	90.86757
C24B9.9	14.12	0.376	0.0165	96.71232	92.10697
C54D10.1	13.56	0.371	0.0246	92.87671	88.45401
gst glutathione S-transferase					
T22G5.2	14.116	0.349	0.0522	96.68493	92.08088
K11G9.6	13.16	0.429	0.0655	90.13698	85.84474
F10D2.9	14.71	0.33	0.0873	100.7534	95.95564
C17H12.8	15.16	0.325	0.1271	103.8356	98.89106
K06A11.1	15.16	0.374	0.1382	103.8356	98.89106
K10D11.1	14.29	0.337	0.1689	97.87671	93.21591
T20G5.7	15.7	0.314	0.1715	107.5342	102.4135
T16G12.1	14.25	0.367	0.1809	97.60273	92.95499
F49A5.6	14.43	0.341	0.4156	98.83561	94.12915
Y49E10.8	15.85	0.291	0.4235	108.5616	103.3920
F38E11.2	14.79	0.383	0.4478	101.3013	96.47749
AC3.7	14.78	0.365	0.848	101.2328	96.41226
UDP-glucuronosyltransferase					
Vector	14.6	0.372			95.23809

Table 3, cont'd

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CF512 (fer-15; fem-1) on RNAi bacteria, whole life, 25C (Jan. 2002) RNAis of Group 2 genes to compare with rrf-3 results (next page). Experiment performed as described above. ZK1251.2 (ins-7) became contaminated--not active?						
	mean	std err	p-value	% vector	% avg. V	
daf-16	11.3	0.279	<0.0001	66.47058	73.71167	
C54G4.6	18.71	0.637	0.0021	110.0588	122.0482	
C04F6.1	14.7	0.586	0.009	86.47058	95.89041	
daf-2	18.13	0.76	0.0509	106.6470	118.2648	
C42D8.2	15.42	0.58	0.0926	90.70588	100.5870	
ZK1251.2	17.05	0.606	0.5162	100.2941	111.2198	
ZK896.8	16.41	0.695	0.7001	96.52941	107.0450	
C32H11.12	16.5	0.635	0.8983	97.05882	107.6320	
vector	17.06	0.574			111.2850	

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Table 3, cont'd

CF596 (fer-15; fem-1; daf-2(mu150)), 25C whole life (w/transient dauer); Jan 2002

Notes: Worms have no self-progeny when raised at 25C due to fer-15 and fem-1 mutations. daf-2(mu150) worms are very long-lived at 25C and pass through a transient dauer state if kept at 25C during late L1, the dauer decision stage.

daf-16 and C05E4.9 (mIs-1) RNAi reduced dauer formation in this experiment;

daf-2 and T10B9.1 RNAi prevented dauer exit at 20C; daf-2 RNAi-treated worms were sick.

(daf-2 nulls are embryonic lethal; very low levels of daf-2 in development may be deleterious) RNAis tested were from Group 1 (upregulated in daf-2(-) conditions, down in daf-16(-)).

Shaded cells are not significantly different from control vector bacteria lifespan (p-value&gt;0.05).

		mean	std err	p-value	% vector	% dauer
C02A12.4	sim. to N-acetylmuraminidase	18.3	0.918	<0.0001	72.619047	100
Daf-16		11.47	0.423	<0.0001	45.515873	33.3
Daf-2		13.88	0.659	<0.0001	55.079365	97.6
F28D1.3	thumatin	18.4	0.927	<0.0001	73.015873	100
T10B9.1	Cyt P450	15.36	0.813	<0.0001	60.952380	97.6
Y54G11A.5b	ctl-2	13.6	0.76	<0.0001	53.968253	84
T20G5.7	unknown	18.63	0.938	0.0008	79.957081	92.6
F38E11.2	hsp-12.6	19.1	0.94	0.0015	75.793650	86
B0213.15	Cyt P450	30	0.833	0.0025	119.04761	100
K11G9.6	mtl-1	19.1	1.1	0.0025	75.793650	92
C05E4.9	malate synthase/isocitrate lyase	19.44	1.048	0.0027	77.142857	60
F10D2.9	fat-7	20.1	1.1	0.0269	79.761904	84
C24B9.4	unknown	19.77	1.064	0.031	78.452380	96.15
K12G11.3	alcohol dehydrogenase	22.57	1.3	0.2898	89.563492	100
vector		25.2	1.3			100

Table 3, cont'd

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CF596 (fer-15; fem-1; daf-2(mu150)), 20C through late L3; shifted to 25 C--rest of life (Mar 2002)  
 Worms must be at 25C in L3 to prevent progeny production through fer-15 and fem-1.  
 Delayed shift until L3 to avoid transient dauer formation. mu150 worms were very long-lived  
 with this protocol. Tested Group 1 RNAis that were not tested previously.

	mean	std err	p-value	% vector
ctl-1 Y54G11A.6	33.622	1.07	<0.0001	87.831765
K07C6.4 CytP450	34.69	0.884	<0.0001	90.621734
daf-16	-16.587	0.8	<0.0001	43.330721
T28B8.2 ins-18	36.02	0.838	0.0002	94.096133
ctl-2 Y54G11A.5b	35.56	0.876	0.0007	92.894461
daf-2	40.62	1.15	0.0009	106.11285
C08A9.1 sod-3	35.45	1.03	0.0163	92.607105
K07E3.3 dao-3	35.75	1.05	0.0196	93.390804
K12G11.4	36.36	1.01	0.1062	94.984326
alcohol dh	38.28	1.12		
vector				



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Table 3, cont'd

CF596 (fer-15; fem-1; daf-2(mu150)), 20C through L3 25C, 25 C rest of life (May 2002)					
Tested additional Group 1 RNAs, repeated genes from first set with this protocol.					
Temp. shift have been slightly earlier than previous experiment, possibly causing shorter lifespan.					
(daf-2 nulls are embryonic lethal; very low levels of daf-2 in development may be deleterious--see Jan CF596 Lifespan)					
	mean	std err	p-value	% vector	
bir-2 C50B8.2	23.335	1.003	<0.0001	81.145460	
ctl-1 Y54G11A.6	23.746	0.864	<0.0001	82.574677	
ctl-1+ctl-2	24.746	0.864	<0.0001	86.052091	
daf-16	15.103	0.344	<0.0001	52.519386	
daf-2	31.163	0.698	<0.0001	108.36665	
T10B9.1	19.792	0.64	<0.0001	68.824981	
ASAH K11D2.2	24.567	0.899	0.0013	85.429634	
K07C6.4	24.421	0.866	0.0015	84.921932	
F38E11.2 hsp-12.6	25.705	0.896	0.0019	89.386931	
C52E4.1 gcp-1	25.64	0.828	0.0031	89.160899	
C46F4.2	25.237	0.882	0.0035	87.759502	
dao-3 K07E3.3	24.137	1.009	0.0038	83.934346	
C06B3.4 17bDH	25.2	0.834	0.0088	87.630837	
ins-18 T28B8.2	25.34	0.842	0.0133	88.117675	
K11G9.6	25.712	0.885	0.015	89.411273	
F11A5.12	25.467	0.832	0.0158	88.559307	
F43D9.4 sip-1	25.435	0.924	0.0203	88.448030	
B0213.15	30.59	0.574	0.0206	106.37410	
K12G11.4	25.903	0.885	0.0384	90.075459	
ctl-2 Y54G11A.5b	25.768	0.923	0.0439	89.606008	
bir-2 apoptosis					
ctl-1					
Cyt P450					
ASAH acid ceramidase					
Cyt P450					
hsp-12.6					
gcp-1 gut cysteine protease					
acyl-CoA synthetase					
dao-3 THF synthetase					
estradiol 17b dh					
ins-18					
mtl-1					
estradiol 17b dh					
sip-1 hsp					
Cyt P450					
alcohol dh					
ctl-2					

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Table 3, cont a

	mean	std err	p-value	% vector
K12G11.3+4	25.634	0.898	0.047	89.140035
T20G5.7	25.574	1.063	0.0677	88.931390
F28D1.3	26.702	0.828	0.0709	92.853913
T27E4.8	26	0.878	0.0782	90.412769
C55B7.4	25.863	0.929	0.0816	89.936363
F10D2.9 fat-7	25.376	1.061	0.1268	88.242862
C24B9.9	28.674	0.896	0.1315	99.711374
ins-7 ZK1251.2	24.518	1.094	0.2468	85.259241
C02A12.4	26.72	0.913	0.2805	92.916507
R12A1.4 ges-1	25.759	0.97	0.3058	89.574712
H22K11.1 asp-3	25.976	0.921	0.3351	90.329311
K12G11.3	28.092	1.005	0.4065	97.687519
F13B12.5	28.08	0.743	0.4685	97.645790
C08A9.1	27.323	0.776	0.4907	95.013388
M04D8.1	25.927	1.056	0.6318	90.158917
AC3.7	27.356	0.912	0.8523	95.128142
K10B3.8	26.717	0.954	0.8786	92.906075
vector	28.757	0.738		

Table 3, cont'd

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***rrf-3* on RNAi bacteria, 20C whole life, Jan 2002**

Note: *rrf-3* is an RNAi-sensitive strain, possibly more active in neurons (not shown yet). Eggs were placed on bacteria and assayed at 20C (*rrf-3* worms are healthy at 20C). Group 2 genes (downregulated in *daf-2* mutants) were tested here.

Shaded cells are not significantly different from control vector bacteria lifespan (p-value>0.05).

*daf-16* RNAi does not seem active in this experiment.

	mean	std err	p-value	% vector
<i>daf-2</i>	31.1	0.749	<0.0001	207.33333
ZK1251.2	23.28	0.512	<0.0001	155.2
C32H11.12	19.7	0.676	0.0001	131.33333
C54G4.6	19.857	0.658	0.0004	132.38
ZK896.8	18.82	0.731	0.0058	125.46666
C42D8.2	18.15	0.576	0.0165	121
C04F6.1	17.47	0.763	0.0881	116.46666
<i>daf-16</i>	14.84	0.476	0.5323	98.93333
vector	15.02	0.613		

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Table 3, cont'd

<i>rrf-3</i> on RNAi bacteria, 20C through late L2, shifted to 25C, back to 20 as Day 1 adults. May 2002					
Note: 25C shift from L2 to Day 1 adult reduces progeny production (almost none) but downshift to 20C allows normal, healthy lifespan ( <i>rrf-3</i> worms are sick when kept at 25C). All genes tested are from Group 2 (downregulated in <i>daf-2</i> mutants).					
		mean	std err	p-value	% vector
C54G4.6 ASMTL	ASMTL/MAF	18.96	0.58	<0.0001	126.4
C32H11.12	unknown	18.66	0.72	<0.0001	124.4
daf-16		11.9	0.372	<0.0001	79.333333
daf-2		28.73	1.57	<0.0001	191.53333
B0024.6	gcy-6	18.92	0.513	<0.0001	126.13333
ZK1251.2 ins-7	ins-7	19.99	1.008	<0.0001	133.26666
K10D11.1	unknown	20.06	0.675	<0.0001	133.73333
C07B5.1 nuc-1	nuc-1 endonuclease	19.56	0.559	<0.0001	130.4
ZK6.10	unknown	19.18	0.799	<0.0001	127.86666
ZK896.8 gcy-18	gcy-18	18.71	0.722	0.0001	124.73333
B0554.6	unknown	18.457	0.643	0.0002	123.04666
C42D8.2 vit-2	vit-2	18.656	0.691	0.0002	124.37333
C32H11.10	unknown	18.25	0.703	0.0004	121.66666
F13B12.5	ins-1	18.28	0.696	0.0004	121.86666
T08G8.10 mtl-2	mtl-2	18.03	0.603	0.0004	120.2
F55G11.5	unknown	17.71	0.763	0.0019	118.06666
F49E12.2	calpain protease	17.47	0.779	0.0048	116.46666
C04F6.1	vit-5	16.45	0.512	0.0058	109.66666
T22G5.2 lbp-7	lbp-7 fatty-acid binding	17.16	0.573	0.0132	114.4
K04E7.2 pep-2	pep-2 oligopeptide transporter	17.06	0.612	0.0165	113.73333

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Table 3, cont'd

	mean	std err	p-value	% vector
C08H9.5 old-1	16.74	0.833	0.0234	111.6
F56G4.2 pes-2	16.77	0.548	0.0598	111.8
ins-21 M04D8.1	16.33	0.551	0.0992	108.86666
T28B8.2 ins-18	15.66	0.746	0.2582	104.4
vector	15	0.608		

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Predicted Gene	Homology	% of Mean Lifespan extension
<b>Chrom. III</b>		
Y37D8A.14	Cytochrome C oxidase	54%
C06E1.10	ATP-dep. RNA helicase (DEAD Box)	31%
K04G7.4	NADH dehydrogenase	28%
K03H1.6	member of Transthyretin-like family	16%
C29F9.7	<i>pat-4, integrin-link kinase</i>	16%
T12D8.2	<i>taf-10</i>	16%
<b>Chrom. IV</b>		
ZK792.2	<i>inx-8, Innexin</i>	44%
Y57G11C.12	NADH-ubiquinone reductase	42%
C28C12.9	<i>gei-9, acyl-CoA dehydrogenase</i>	29%
T21D12.4	<i>pat-6, Parvin a</i>	13%
<b>Chrom. V</b>		
T05H4.12		80%
C06H2.1	ATP synthase D chain	49%
C53A5.1		45%
K10D6.2		35%
C14C10.3		23%
W02G9.5		15%
T06C12.13	Zink finger, C4-type steroid hormone receptor	50%
K03D7.8		15%
F26F2.1		15%
<b>Chrom. X</b>		
F02E8.1	ATP Synthase F0	20%
C49F5.1	S-adenosyl methionine synthatase	14%
T03G11.8	<i>zig-6, Immunoglobulin superfamily</i>	10%

Table 5

Class 1: Genes upregulated under *daf-2(-)* conditions

Cosmid no.	Gene	Brief description	Per cent of vector control lifespan (experiment)				Canonical GTAAU/cA	New CTTATCA
	<i>daf-16</i>		43.3(b)*	52.5(c)*	52.5(d)*	38.8(j)*	—	—
Y54G11A.5b	<i>ctl-2</i>	Peroxisomal catalase	54(a)*	92.9(b)†	89.6(c)†	84.8(j)†	1	3
T10B9.1	<i>dod-1</i>	Cytochrome P450 family, low similarity to mouse cytochrome P450 Cyp3a11	61.0(a)*	68.8(c)*			3	1
T27E4.8	<i>hsp-16.1</i>	Member of the <i>C. elegans</i> <i>hsp-16</i> family; identical <i>hsp-16.11</i>	71.3(d)†				0	0
C02A12.4	<i>lys-7</i>	Response to pathogenic bacteria; lysosyme/similar to N-acetylmuraminidase	72.6(a)*	92.9(c)‡	79.8(d)†	56.1(e)*	2	1
F28D1.3	<i>dod-2</i>	Thaumatococcus plant pathogenesis associated (PR) proteins, similar to F28D1.5	73.0(a)*	92.9(c)‡	92.2(j)‡		2	2
F38E11.2	<i>hsp-12.6</i>	Hsp20/alpha crystalline family, similar to alpha-B crystalline	75.8(a)†	89.4(c)†			3	2
K11G9.6	<i>mtl-1</i>	Metallothionein-related cadmium-binding protein	75.8(a)†	89.4(c)†			2	3
C05E4.9	<i>gal-7</i>	Malate synthase family/isocitrate lyase family	77.1(a)†				5	3
C24B9.9	<i>dod-3</i>	Unknown protein	78.5(a)†	99.7(c)‡			6	1
F32A5.5	<i>dod-4</i>	Aquaporin AQP; major intrinsic protein (MIP) family of transmembrane channels	78.7(d)*				1	4
T22G5.7	<i>dod-5</i>	Saposin type B	79.2(d)*	79.7(e)†			4	1
F10D2.9	<i>fat-7</i>	Putative stearyl-CoA delta-9 fatty acid desaturase/polyunsaturated fatty acid biosynthesis	79.8(a)†	88.2(c)‡	94.9(j)‡		3	1
T20G5.7	<i>dod-7</i>	Medftrin-like ShK toxin	80.0(a)†	88.9(c)‡	82.3(d)†	72.3(e)‡	1	2
T27E4.9	<i>hsp-16.49</i>	Hsp20/alpha crystallin family, similar to alpha-B crystalline	81.0(d)†				4	1
C50B8.2	<i>blr-2</i>	Protein with two baculoviral inhibitor of apoptosis protein repeat (BIR) domains	81.1(c)*				5	1
T27E4.2	<i>hsp-16.11</i>	Member of the <i>C. elegans</i> <i>hsp-16</i> family; identical to <i>hsp-16.11</i>	81.4(d)†				1	0
T20G5.8		Medftrin-like ShK toxin	84.3(d)†	70.0(e)†	97.5(j)‡		1	2
T07C4.4	<i>spp-1</i>	Saposin; similar to bactericidal amoebapores, may act as an antibacterial agent	84.3(d)†	77.0(e)†			1	2
K11D2.2	<i>dod-7</i>	ASAH acid ceramidase; cholesteryl glycerol hydrolase, cleaves C-N non-peptide bonds in linear amides	85.4(c)†				1	3
C06B3.4	<i>dod-8</i>	Estradiol 17b dh; short-chain dehydrogenase-reductase family oxidoreductases	87.6(c)‡				4	1
Y54G11A.6	<i>ctl-1</i>	Cytosolic catalase	87.8(b)*	82.6(c)*	82.2(j)*		3	0
C46F4.2	<i>dod-9</i>	Acyl-CoA synthetase; high similarity to long-chain fatty acid-CoA ligase 4	87.8(c)†				2	1
F43D9.4	<i>slp-1</i>	Hsp20/alpha crystallin family, moderately similar to <i>C. elegans</i> HSO-16 involved in heat shock response	88.4(c)†				3	1
F11A5.12	<i>dod-10</i>	Short-chain dehydrogenase-reductase family, NAD- or NADP-dependent oxidoreductases	88.6(c)†				1	3
C52E4.1	<i>gcp-1</i>	Cysteine protease expressed in the intestine	89.2(c)‡	92.9(j)‡			1	0
K12G11.3	<i>dod-11</i>	High similarity to <i>C. albicans</i> Adh1p, an alcohol dehydrogenase	89.6(a)‡	97.7(c)‡			3	2
R12A1.4	<i>ges-1</i>	Carboxylesterase expressed in gut cells	89.6(c)‡				1	3
C55B7.4	<i>dod-12</i>	Short branched chain acyl-CoA dehydrogenase (human ACADSB)	89.9(c)†				0	1
H22K11.1	<i>asp-3</i>	Probable aspartyl protease and an orthologue of human cathepsin D	90.3(c)‡				3	0
Y46H3A.3	<i>hsp-16.2</i>	Strong similarity to <i>C. elegans</i> HSP-16 heat shock protein, Hsp20/alpha crystallin family	90.4(d)‡				1	0
K07C6.4	<i>dod-13</i>	Cytochrome P450 family, low similarity to cytochrome P450 subfamily 2C polypeptide 8	90.6(b)*	84.9(c)†			1	2
R03E9.1	<i>mdf-1</i>	MAD family of putative transcription factors, interacts with <i>C. elegans</i> MAX-1	91.1(d)†				1	2
C08A9.1	<i>sod-3</i>	Manganese superoxide dismutase	92.6(b)†	95.0(c)‡	83.2(j)*		6	2
K10B3.8	<i>gpd-2</i>	Glyceraldehyde-3-phosphate dehydrogenase	92.8(c)‡				4	0
K07E3.3	<i>dao-3</i>	Tetrahydrofolate dehydrogenase/cyclohydrolase catalytic domain, NAD(P)-binding domain	93.4(b)†	83.9(c)†	95.6(j)‡		5	2
T28B8.2	<i>ins-18</i>	Insulin-like protein of the type-beta subfamily; may be a ligand for the DAF-2 receptor	94.1(b)†	88.1(c)†			2	1
K12G11.4	<i>dod-14</i>	High similarity to <i>C. albicans</i> Adh1p alcohol dehydrogenase; Zn alcohol dehydrogenase family	95.0(b)‡	90.1(c)†			4	1
AC3.7	<i>dod-15</i>	UDP-glucuronosyl, UDP-glucosyl transferase domains	95.1(c)‡				4	2
	<i>daf-2</i>		106.1(b)†	108.4(c)*	115.2(j)†		—	—
B0213.15	<i>dod-16</i>	Cytochrome P450, oxidation of arachidonic acid to eicosanoids; (mouse Cyp2j5)	119.0(a)†	108.4(c)†			1	2

The table is a summary of data from selected class 1 genes. Animals were treated with RNAi of selected genes and lifespans were compared to those of animals treated with control vector RNAi; experiments are briefly described below. "dod" stands for "downstream of DAF-16". (Detailed lifespan data are included in the Supplementary Information.) The number of canonical DAF-16 and new sequences in the 5 kb upstream of each gene is also shown. All experiments were performed with  $n \geq 60$  animals. (a), *daf-2(mu150)*, 25 °C whole life; (b), *daf-2(mu150)* shifted from 20 °C to 25 °C at L3; (c), *daf-2(mu150)* shifted from 20 °C to 25 °C at L2; (d), *daf-2(mu150)* shifted from 20 °C to 25 °C at L4; (e), *daf-2(e1370)* shifted from 20 °C to 25.5 °C at L4; (f), *mtl-3(pk1426)*; *daf-2(e1370)* at 20 °C. \* $P \leq 0.0001$ ; † $P \leq 0.001$ ; ‡ $P \leq 0.005$ ; § $P \leq 0.01$ ; ¶ $P \leq 0.05$ ; || $P > 0.05$ .

Table 6

Class 2: Genes downregulated under <i>daf-2(-)</i> conditions						
Cosmid no.	Gene	Brief description	Per cent of vector control lifespan experiment			New CTTATCA
	<i>daf-2</i>		130.5(a) <sup>f</sup>	207 (b) <sup>*</sup>	191.5(c) <sup>*</sup>	-
K10D11.1	<i>dod-17</i>	DUF141 domain of unknown function, high similarity to uncharacterized <i>C. elegans</i> F55G11.8	133.7(c) <sup>*</sup>			4
C07B5.5	<i>nuc-1</i>	Endonuclease with strong similarity to <i>H. sapiens</i> DNase II; DNA degradation during apoptosis	130.4(c) <sup>*</sup>	101.9(d) <sup>g</sup>		2
C54G4.6	<i>dod-18</i>	Mat-like protein family, inhibitors of septum formation, low similarity to uncharacterized <i>S. pombe</i> Spac3g6.03cp	129.2(a) <sup>*</sup>	132.4(b) <sup>f</sup>	126.4(c) <sup>*</sup>	0
ZK6.10	<i>dod-19</i>	Protein of unknown function	127.9(c) <sup>*</sup>			1
B0024.6	<i>gcy-6</i>	Putative guanylyl cyclase expressed in the ASEL neuron	126.1(c) <sup>*</sup>			2
B0554.6	<i>dod-20</i>	Protein of unknown function (DUF274) family, high similarity to uncharacterized <i>C. elegans</i> ZK6.11	123.0(c) <sup>*</sup>			3
C32H11.10	<i>dod-21</i>	DUF141 domain of unknown function, strong similarity to uncharacterized <i>C. elegans</i> C32H11.9	121.7(c) <sup>f</sup>			2
C04F6.1	<i>vit-5</i>	Vitellogenin; 170 kDa yolk protein	121.5(a) <sup>f</sup>	116.5(b) <sup>g</sup>	109.7(c) <sup>g</sup>	1
T08G5.10	<i>mtl-2</i>	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> MTL-1	120.2(c) <sup>f</sup>	96.5(d) <sup>g</sup>		1
F55G11.5	<i>dod-22</i>	DUF141 domain of unknown function, high similarity to uncharacterized <i>C. elegans</i> K10D11.2	118.1(c) <sup>g</sup>			3
F49E12.2	<i>dod-23</i>	Protein of unknown function	116.5(c) <sup>f</sup>	101.1(d) <sup>g</sup>		1
T22G5.2	<i>lbp-7</i>	High similarity to <i>C. elegans</i> LBP-5 (locomotory behaviour) lipocalin and cytosolic fatty-acid binding	114.4(c) <sup>g</sup>	101.4(d) <sup>g</sup>		0
K04E7.2	<i>pep-2</i>	Member of the proton-coupled oligopeptide transporter superfamily	113.7(c) <sup>g</sup>			3
ZK1251.2	<i>ins-7</i>	Insulin-like protein of the type-beta subfamily	155.2(b) <sup>*</sup>	133.3(c) <sup>*</sup>		0
F56G4.2	<i>pes-2</i>	Unknown function, has very strong similarity to uncharacterized <i>C. elegans</i> F56G4.3	111.8(c) <sup>g</sup>	124.5(d) <sup>g</sup>		3
C08H9.5	<i>old-1</i>	Putative receptor tyrosine protein kinase; similar to human and <i>D. melanogaster</i> FGF receptor protein kinases	111.6(c) <sup>g</sup>	109.6(d) <sup>g</sup>		0
C32H11.12	<i>dod-24</i>	DUF141 domain of unknown function, high similarity to uncharacterized <i>C. elegans</i> C32H11.9	131.3(b) <sup>*</sup>	124.4(c) <sup>*</sup>		2
ZK896.8	<i>gcy-18</i>	Guanylate cyclase catalytic domain; receptor family ligand binding and protein kinase domain	125.5(b) <sup>g</sup>	124.7(c) <sup>*</sup>		3
C42D8.2	<i>vit-2</i>	Vitellogenin structural genes (yolk protein genes)	121.0(b) <sup>g</sup>	124.4(c) <sup>f</sup>	1	1
	<i>daf-16</i>		79.3(c) <sup>*</sup>			-

The Table is the same as Table 1, except for class 2 genes (see Table 1 legend). (f), *fer-15(b26)*; *fam-1(hcl7)* at 25 °C whole life; (g), *mf-3(pk1426)* at 20 °C whole life; (h), *mf-3(pk1426)* shifted to 25 °C at L2-L4, back to 20 °C; (i), *mf-3(pk1426)* shifted to 25 °C at L2-L4, back to 20 °C

\*P ≤ 0.0001; †, P ≤ 0.001; ‡, P ≤ 0.005; §, P ≤ 0.01; ¶, P ≤ 0.05; ||, P > 0.05



## 1s.) Class 1 and Class 2 Genes From Microarray Analysis

## (a) Class 1 Genes

6R55.1a	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> 6R55.1A
AC3.7	Protein containing a UDP-glucuronosyl and UDP-glucosyl transferase domain, which transfer glycosyl groups to small hydrophobic molecul
B0213.15*	Member of the cytochrome P450 family, has low similarity to cytochrome P450 subfamily IJ5 (mouse Cyp2j5), which is a heme-thiolate pr
B0238.1	Member of the carboxylesterase type B family, has low similarity to carboxylesterase 1 (monocyte-macrophage serine esterase 1, rat Cest1
B0507.8	B0507.8 Protein of unknown function, has low similarity to uncharacterized <i>C. elegans</i> B0507.9
C01H6.6	Protein containing a DUF21 domain of unknown function, has weak similarity to a region of <i>S. cerevisiae</i> Ami3p, which is required for norm
C02A12.4*	lys-7; C02A12.4 Protein likely involved in the response to pathogenic bacteria
C05C12.4*	Protein of unknown function
C05E4.9*	gel-7; C05E4.9 Member of the malate synthase family and the isocitrate lyase family, which catalyze conversion of isocitrate to succinate s
C06B3.4	Member of the short-chain dehydrogenase-reductase family, which are oxidoreductases, has moderate similarity to hydroxysteroid 17-beta
C06G8.1	Member of the MN3-saliva family, which contain a region of two transmembrane helices
C08A9.1	sod-3; MnSOD; C08A9.1 Manganese superoxide dismutase
C08B6.4	Member of the plant chitinase class I family, which hydrolyze the beta-1,4-N-acetyl-D-glucosamine bonds in chitin polymers destroying chi
C08E3.6**	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme, and a DUF38 dom
C08E3.4**	Protein containing a DUF38 domain of unknown function, has low similarity to a region of <i>C. elegans</i> T07D3.1, which functions in gametog
C08E8.4	C08E8.4: Protein of unknown function, has weak similarity to <i>C. elegans</i> C07G3.2
C08F11.11	Protein of unknown function, has moderate similarity to uncharacterized <i>C. elegans</i> Y45F10C.2
C08F11.3	C08F11.3: Protein of unknown function, has moderate similarity to <i>C. elegans</i> F56G4.1
C09G12.8a	ced-10; C09G12.8B; rac-1; C09G12.8 Member of a class of genes that include ced-2 and ced-5 that control cell-corps removal, member i
C10C5.2	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme
C10G8.4	Protein containing a trypsin inhibitor-like cysteine rich domain, has moderate similarity to uncharacterized <i>C. elegans</i> Y69H2.5
C13B9.1	C13B9.1 C13B9.1
C15H9.1	Putative nicotinamide nucleotide transhydrogenase
C17G1.4*	Protein of unknown function, has weak similarity to a region of mucin (episialin, human MUC1), which is a cell surface transmembrane glyco
C17G10.5	lys-8; C17G10.5 Protein likely involved in the response to pathogenic bacteria
C17H12.11	C17H12.11: Protein of unknown function, has weak similarity to <i>C. elegans</i> Y106G6D.4
C18A11.1	C18A11.1: Protein of unknown function, has weak similarity to <i>C. elegans</i> T24C4.3
C24A11.8a	Protein containing two FERM domains (Band 4.1 family), has weak similarity to erythrocyte membrane protein band 4.1 (human EPB41), v
C24B9.9	C24B9.9: Protein of unknown function, has moderate similarity to <i>C. elegans</i> T04C12.1
C25E10.8	Protein containing two trypsin inhibitor-like cysteine rich domains, has high similarity to uncharacterized <i>C. elegans</i> C25E10.9
C25E10.9	Protein containing two trypsin inhibitor-like cysteine rich domains, has high similarity to uncharacterized <i>C. elegans</i> C25E10.8
C26C6.3*	Member of the astacin (M12A) family of metalloproteases, contains a CUB domain and a type 1 thrombospondin domain, has low similarity
C27D6.10	srb-1; C27D6.10 G protein-coupled receptor; member of a subfamily with SRB proteins, which are expressed in chemosensory neurons; n
C34C6.7*	C34C6.7: Protein of unknown function
C34D10.2**	Protein containing three CCHH-type zinc finger domains, which bind DNA or RNA
C37H5.2	Member of the alpha or beta hydrolase fold family, has weak similarity to <i>S. cerevisiae</i> Ict1p, which is required for normal sensitivity to cop
C40H1.5*	Member of the transferrin-like family, has high similarity to uncharacterized <i>C. elegans</i> T28B4.3
C40H5.1	Protein of unknown function, has strong similarity to uncharacterized <i>C. elegans</i> ZC412.7
C44H9.5	C44H9.5: Protein of unknown function
C46H11.2*	Member of the flavin-binding monooxygenase family, which are xenobiotic-metabolizing enzymes, has low similarity to flavin containing mo
C48B6.9	C48B6.9: Protein of unknown function
C49G7.5	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> C49G7.10
C49G7.7	Protein containing a DUF141 domain of unknown function
C50F7.2	Protein of unknown function, has low similarity to a region of aggrecan 1 (large aggregating chondroitin sulfate proteoglycan, human AGC1
C50F7.5	Protein of unknown function, has low similarity to a region of heavy polypeptide of neurofilament (mouse Nft), which is a structural protein
C50F7.10*	Member of the glycosyl hydrolases family 1, which may be involved in carbohydrate metabolism, has low similarity to a region of lactase-pl
C52E4.1	gcp-1; cpr-1; C52E4.1 Cysteine protease expressed in the intestine
C53B7.3	C53B7.3: Protein with weak similarity to EGF-like repeats, has moderate similarity to <i>C. elegans</i> F46C8.4
C54C8.9*	Protein of unknown function

C54D10.1	Protein with high similarity to <i>C. elegans</i> CDR-1, which is a cadmium-inducible lysosomal protein required for resistance to cadmium toxicity
C54D10.3	Protein of unknown function, has moderate similarity to uncharacterized <i>C. elegans</i> C46H11.7
C54D10.7*	Protein of unknown function
C55A6.8	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i>
C56A3.2*	Member of the transthyretin-like family, has high similarity to uncharacterized <i>C. elegans</i> T07C12.7
E01A2.8	Member of the arylesterase family, which catalyze hydrolysis of organophosphorus esters, has a region of low similarity to paraoxonase 2
E01G4.3	E01G4.3: Protein of unknown function
E04F6.8	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> E04F6.9
E04F6.9	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> E04F6.8
F07H5.4	F07H5.4: Protein of unknown function
F08B1.1	F08B1.1: vhp-1: Member of the dual specificity phosphatase, catalytic domain protein family
F08B12.4*	F08B12.4; XM28 Protein of unknown function
F09F7.6*	F09F7.6: Protein of unknown function
F09F7.7*	F09F7.7; F09F7.7A Protein containing a 2OG-Fe(II) oxygenase superfamily domain, has low similarity to uncharacterized human FLJ2001
F11A5.12	Member of the short-chain dehydrogenase-reductase family, which are NAD- or NADP-dependent oxidoreductases, has moderate similarity
F12A10.7	F12A10.7: Protein of unknown function
F15B9.1	far-3; F15B9.1 Protein of unknown function, has moderate similarity to uncharacterized <i>C. elegans</i> F02A9.2
F15E6.8	F15E6.8: Protein of unknown function, has weak similarity to <i>C. elegans</i> F15E6.4
F16H6.7	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> F16H6
F17B5.1*	Protein of unknown function, has a region of moderate similarity to <i>C. elegans</i> R05H5.3, which is a putative nuclear thioredoxin involved in
F21C10.10*	Protein of unknown function
F21F3.3	Member of the isoprenylcysteine carboxyl methyltransferase (ICMT) family, has moderate similarity to isoprenylcysteine carboxyl methyltra
F28D1.3	Member of the thaumatin family of plant pathogenesis associated (PR) proteins, has strong similarity to uncharacterized <i>C. elegans</i> F28D1
F28D1.5	Member of the thaumatin family of plant pathogenesis associated (PR) proteins, has strong similarity to uncharacterized <i>C. elegans</i> F28D1
F28F8.2*	Member of the AMP-binding enzyme family, has weak similarity to <i>S. cerevisiae</i> Fat2p, which is a peroxisomal AMP-binding protein
F32A5.5*	Member of the major intrinsic protein (MIP) family, which are transmembrane channels, has moderate similarity to <i>C. elegans</i> AQP, which
F35D11.11**	Protein of unknown function
F36F2.2	F36F2.2: Protein of unknown function
F38E11.1*	F38E11.1; hsp12.3 Member of the Hsp20 or alpha crystallin family, has moderate similarity to a region of alpha-B crystallin (mouse Cryab)
F38E11.2*	hsp-12.6; hsp12.8; F38E11.2 Member of the Hsp20 or alpha crystallin family, has a region of moderate similarity to a region of alpha-B cry
F40D4.3**	Member of the 7-transmembrane chemoreceptor family of G protein-coupled receptors (GPCR), has high similarity to uncharacterized <i>C. e</i>
F40F12.1	Member of the transthyretin-like family, has very strong similarity to uncharacterized <i>C. elegans</i> K03H1.3
F42F12.6	Protein of unknown function, has very strong similarity to uncharacterized <i>C. elegans</i> F42F12.1
F45D3.4	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> F45D3
F45E6.5	F45E6.5: Protein of unknown function
F46C5.1*	Protein of unknown function
F47H4.10*	skr-5; F47H4.10; F47H4.F Protein with high similarity to <i>C. elegans</i> SKR-1, member of the Skp1 family, which are required for targeted pro
F48D6.4*	F48D6.4: Protein of unknown function
F49A5.6	Member of the thaumatin family of plant pathogenesis associated (PR) proteins, has high similarity to uncharacterized <i>C. elegans</i> F28D1.2
F49E11.9	Member of the SCP-like extracellular protein family, has low similarity to a region of <i>S. cerevisiae</i> Pry3p, which may have a role in mating c
F52A8.2**	eat-11; gbp-2; Beta subunit of G protein; member of the WD repeat protein family
F53A9.1*	Protein of unknown function
F53C3.5**	Protein containing a CX module domain, has high similarity to uncharacterized <i>C. elegans</i> F53C3.5
F53G12.6**	Protein containing one protein kinase domain and one Src homology 2 (SH2) domain, which are regulatory modules of intracellular signal
F53F4.13	F53F4.13: Protein of unknown function, has weak similarity to <i>C. elegans</i> F20A1.10
F54D5.3*	Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> F54D5.4
F54F7.3	F54F7.3: Protein of unknown function
F57H12.7	meo-17; F57H12.7 Protein that is expressed in touch receptor neurons and is required for mechanosensory perception
F58B3.4	Protein of unknown function, has low similarity to uncharacterized human C20orf3
F58B3.6	Protein with low similarity to interferon-related developmental regulator (rat Irf1), which plays a role in muscle differentiation and is induce
F58F9.7	Member of the acyl-CoA oxidase family, contains an acyl-CoA dehydrogenase C-terminal domain, has moderate similarity to acyl Coenzym
H04J21.1	Protein containing a WSN domain of unknown function, has moderate similarity to uncharacterized <i>C. elegans</i> R155.4
H10D18.2	Member of the SCP-like extracellular protein family, has low similarity to protease inhibitor 15 (human PI15), which is a trypsin inhibitor inw

H10D18.3	Protein of unknown function, has moderate similarity to uncharacterized <i>C. elegans</i> R08F11.5
H12D21.1	H12D21.1: Protein of unknown function, has weak similarity to <i>C. elegans</i> ZC412.7
H14N18.1	H14N18.1: unc-23; Highly similar to mammalian BAG-2, BCL2-associated athanogene 2, a chaperone regulator
H16D19.1*	H16D19.1; T07D10.4 Member of the C-type lectin protein family
H22K11.1	asp-3; H22K11.1 Probable aspartyl protease and an ortholog of human cathepsin D
H23N18.2	Protein containing a UDP-glucuronosyl and UDP-glucosyl transferase domain, which transfer glycosyl groups to small hydrophobic molecules
H24O09.f*	gel-1; C05E4.9; Member of the malate synthase family and the isocitrate lyase family, which catalyze conversion of isocitrate to succinate
H24O09.e*	gel-1; C05E4.9; Member of the malate synthase family and the isocitrate lyase family, which catalyze conversion of isocitrate to succinate
H24O09.a*	gel-1; C05E4.9; Member of the malate synthase family and the isocitrate lyase family, which catalyze conversion of isocitrate to succinate
H24O09.c	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme, has moderate similarity
JC8.8	Member of the transthyretin-like family, has high similarity to uncharacterized <i>C. elegans</i> C56A3.2
K02B7.1	K02B7.1: Member of the polypeptide chain release factor protein family
K03H1.4	Member of the transthyretin-like family, has high similarity to uncharacterized <i>C. elegans</i> K03H1.3
K07A1.7	K07A1.7: Protein with similarity to <i>D. melanogaster</i> HDC (headcase) protein, a branching inhibitor produced by specialized tracheal cells
K07C6.4*	Member of the cytochrome P450 family, has low similarity to cytochrome P450 subfamily 2C polypeptide 6 (mephenytoin 4-hydroxylase, h)
K08B4.6	Protein containing a cystatin domain, has high similarity to uncharacterized <i>C. elegans</i> R01B10.1
K08F4.7	gst-4; K08F4.7; CeGST1 Protein containing glutathione S-transferase N-terminal and C-terminal domains, has moderate similarity to prost
K08H2.6	hpl-1; K08H2.6 Protein containing a Chromo shadow domain and a Chromo domain, which bind chromatin, has moderate similarity to chrc
K09C4.5	Member of the sugar (and other) transporter family, has weak similarity to <i>C. elegans</i> C35A11.4
K10B3.8	gpd-2; K10B3.8 Glyceraldehyde-3-phosphate dehydrogenase
K10D2.5	K10D2.5: Protein of unknown function
K10D2.7	Member of the uncharacterized DUF170 (Ancient conserved region (ACR), COG2104) family
K10E9.1	K10E9.1: Protein of unknown function
K11D2.2	Member of the cholesteryl glycolipase family, which cleave carbon-nitrogen non-peptide bonds in linear amides, has moderate similarity
K11G9.6*	mtl-1; CeMT-1; mtl-1; mtl-1; K11G9.6 Protein of unknown function, has high similarity to uncharacterized <i>C. elegans</i> MTL-2, which is a likely
K12G11.3*	Protein with high similarity to <i>C. albicans</i> Adh1p, which is an alcohol dehydrogenase and may function as an extracellular matrix adhesin,
K12G11.4	Protein with high similarity to <i>C. albicans</i> Adh1p, which is an alcohol dehydrogenase that may also function as an extracellular matrix adhe
M02D8.4	Protein with high similarity to asparagine synthetase ( <i>S. cerevisiae</i> Asn1p), which acts in nitrogen metabolism, member of the asparagine s
M60.3	M60.3: Protein of unknown function
R03E9.1	mdl-1; R03E9.1 Member of the MAD family of putative transcription factors, interacts with <i>C. elegans</i> MAX-1
R09B5.6*	Protein with high similarity to short chain L-3-hydroxyacyl-Coenzyme A (human HADHSC), which catalyzes the penultimate step in the fatt
R09B5.9	R09B5.9: Protein of unknown function
R13F6.2**	Protein of unknown function, has strong similarity to uncharacterized <i>C. elegans</i> R13F6.8
T01C3.10	nmr-2; T01C3.10 Protein containing a ligand-gated ion channel domain, which are found in ionotropic glutamate receptors and NMDA rece
T02B5.1*	Member of the carboxylesterase type B family, has low similarity to carboxylesterase 1 (monocyte-macrophage serine esterase 1, liver est
T06E6.5	Protein containing two DUF38 domains of unknown function and an F-box domain, which serves as a link between a target protein and a l
T07D10.4*	T07D10.4: Member of the C-type lectin protein family
T10B9.1	Member of the cytochrome P450 family, has low similarity to cytochrome P450 subfamily 3a polypeptide 11 (mouse Cyp3a11), which is a
T17A3.3	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme, has high similarity
T19B10.2	T19B10.2: Protein of unknown function
T20G5.7*	T20G5.7: Protein of unknown function, has moderate similarity to <i>C. elegans</i> T20G5.8
T21C9.13	T21C9.13: Protein with strong similarity to <i>C. elegans</i> T21C9.11 gene product
T21C9.8	Member of the transthyretin-like family, has high similarity to uncharacterized <i>C. elegans</i> F09F3.6
T22F3.11*	Member of the sugar (and other) transporter family, has weak similarity to solute carrier family 17 member 1 (human SLC17A1), which is li
T22G5.7	T22G5.7: Protein with weak similarity to <i>C. elegans</i> T07C4.4 (Protein with similarity to bactericidal amoebapores that may act as an antiba
T23B3.2	Member of the uncharacterized protein family UPF0057 T23B3.2: Protein of unknown function, has weak similarity to <i>C. elegans</i> F47B7.1
T23G7.3	Protein containing a G-patch domain, which are found in RNA processing proteins, has low similarity to uncharacterized p12-TRF1-Interac
T23H2.2**	Protein containing two C2 domains, has moderate similarity to synaptotagmin 4 (rat Syt4), which regulates the kinetics of fusion pore open
T24D1.3	Protein of unknown function, has low similarity to uncharacterized <i>C. elegans</i> T23F6.3 T24D1.2: Protein with strong similarity to <i>C. elegans</i>
T24H7.3	T24H7.3: Protein of unknown function, has moderate similarity to <i>C. elegans</i> Y43F8B.M
T25C12.2	Protein of unknown function, has a region of moderate similarity to a region of <i>C. elegans</i> T08A9.8, which is an antibacterial peptide
T26F2.1	Member of the plant chitinase class I family, which hydrolyze the beta-1,4-N-acetyl-D-glucosamine bonds in chitin polymers destroying cell
T27E4.8	hsp-16; hsp-16.1; (hsp-16A); hsp-16A1; (hsp16-1); hsp16-1b; T27E4.8; (T27E4.2) Member of the <i>C. elegans</i> hsp-16 family; identical to hsp

T28B8.2*	Ins-18; Celsulin-1; Insulin-like protein of the type-beta subfamily, may be a ligand for the DAF-2 receptor
T28F4.5*	Protein of unknown function, has high similarity to uncharacterized C. elegans F54B8.4
VC5.3	Protein that is positively regulated by DBL-1
VZK822L.1	fat-5; VZK822L.1 Putative stearyl-CoA delta-9 fatty acid desaturase involved in polyunsaturated fatty acid (PUFA) biosynthesis, specific to
W01B11.6**	Member of the thioredoxin family, which are small enzymes involved in redox reactions
W02D3.1*	Protein containing a heme-binding domain, has moderate similarity to mitochondrial outer membrane (OM) ferredoxin b(5) (rat omf)
W03F11.3*	not found
W03F11.1	Protein containing two chitin binding peritrophin-A domains, which contain six conserved cysteines that probably form three disulfide bridges
W05H9.1	Protein of unknown function
W06D12.3	fat-5; W06D12.3 Putative fatty acid desaturase, involved in polyunsaturated fatty acid (PUFA) biosynthesis
W08D2.4*	fat-3; W08D2.4 Putative front end desaturase, involved in polyunsaturated fatty acid (PUFA) biosynthesis
W09G10.4	Protein containing two clathrin-associated adaptor N-terminal domains, which are components of coated vesicles, has moderate similarity to
W10G6.3	ita-2; W10G6.3; Cef-IF-A2 Member of the intermediate filament family, which are components of the cytoskeleton and nuclear envelope, $\alpha$
Y105C5A.12*	Y105C5A.12: Protein of unknown function
Y15E3B.f	Y15E3B.f; Y15E3B.f
Y40B10A.6	Member of the type 3 O-methyltransferase family, has high similarity to uncharacterized C. elegans Y32B12A.3
Y43C5A.3**	Protein of unknown function
Y46C8AR.1*	Y46C8AR.1: Protein containing a C-type lectin domain, which mediate calcium-dependent carbohydrate recognition, has high similarity to
Y46H3A.3	hsp-16.2; Y46H3A.3; Y46H3A.3 Protein with strong similarity to C. elegans HSP-16, which is a heat shock protein, member of the Hsp20
Y47H9C.1	Member of the protein of unknown function (DUF274) family, has high similarity to uncharacterized C. elegans ZK6.11 family
Y51A2B.1	Y51A2B.1: Protein of unknown function, has moderate similarity to C. elegans C07G3.2
Y51A2D.11	Member of the transferrin-like family, has high similarity to uncharacterized C. elegans F09F3.8
Y51B9A.8	Y51B9A.8: Protein with weak similarity to C. elegans F15A4.6
Y53F4B.14	Y53F4B.14: Protein with weak similarity to C. elegans Y53F4B.Q
Y57G11C.14*	Protein of unknown function, has strong similarity to uncharacterized C. elegans ZK637.12
Y6E2A.3	Y6E2A.3: Protein of unknown function, has weak similarity to C. elegans Y6E2A.5
ZC395.5	ZC395.5: Protein of unknown function
ZK1320.2	ZK1320.2: Protein of unknown function, putative paralog of C. elegans ZK1320.3
ZK270.2a	Protein containing six FERM domains (Band 4.1 family), which link cytoplasmic proteins to membranes, has a region of low similarity to a
ZK355.3*	ZK355.3: Protein of unknown function
ZK384.1	Member of the SCP-like extracellular protein family, has low similarity to uncharacterized C. elegans ZK384.2
ZK384.3	Member of the aspartyl (acid) protease family, which include pepsins, cathepsins, and renins, has a region of high similarity to C. elegans
ZK507.3	Protein of unknown function, has high similarity to uncharacterized C. elegans ZK507.1
ZK973.7	ZK973.7: Protein of unknown function, putative paralog of C. elegans ZK973_14.1

\*: Genes that also scored significantly in SAM analysis

\*\* : Genes with lower fold-difference but consistently upregulated (identified through SAM analysis)

In the set of mutant arrays, 60 upregulated and 54 downregulated genes were found to be significant;  
q-value = 0.0011197 with 0.6207 median false significant genes, at delta = 1.58.

Annotations are from WormPD ([www.incyte.com/proteome/WormPD](http://www.incyte.com/proteome/WormPD))

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## (b) Class 2 Genes

B0281.5	Protein containing a K <sup>+</sup> channel tetramerization domain, which facilitate assembly of alpha-subunits into functional tetrameric channels, has
B0365.6*	B0365.6: Member of the C-type lectin protein family
B0478.1	lck-1; B0478.1A; B0478.1 Neuronally expressed serine/threonine protein kinase of the MAP kinase subfamily
B0554.6	Member of the protein of unknown function (DUF274) family, has high similarity to uncharacterized C. elegans ZK6.11
C01B7.1*	Protein containing four C2H2 type zinc finger domains, which bind nucleic acids
C04F12.3	lck-1; C04F12.3 Protein containing six ankyrin (Ank) repeats and a death domain, which may mediate protein-protein interactions, has a re
C04F6.1	C04F6.1: vit-6: 170 kDa yolk protein
C08F11.8	Protein containing a UDP-glucuronosyl and UDP-glucosyl transferase domain, has low similarity to UDP glucosyltransferase 1 A3 (human
C08F8.5*	Protein containing two F-box domains, which serve as a link between a target protein and a ubiquitin-conjugating enzyme
C09G1.1**	Protein of unknown function
C12C8.2*	Member of the cysteine-methionine metabolism pyridoxal-phosphate-dependent enzyme family, has moderate similarity to cystathionase (
C16C10.5**	Protein of unknown function, has high similarity to uncharacterized human FLJ11089
C17B7.1	Member of the 7-transmembrane chemoreceptor family of G protein-coupled receptors (GPCR), has strong similarity to uncharacterized C
C17H12.8	Protein containing a DUF141 domain, has high similarity to uncharacterized C. elegans F08G5.6
C18A3.2*	Member of the ZIP zinc transporter family, which may be metal transporters, has low similarity to uncharacterized C. elegans F30B5.7
C13A3.10**	Protein of unknown function
C25B8.3	cpr-6; C25B8.3A; C25B8.3 Member of the Cathepsin B-like Cysteine Protease family
C25F6.3	Protein with high similarity to dihydropyrimidine dehydrogenase (rat Dpyd), member of the pyridine nucleotide-disulfide oxidoreductase and
C31A11.5	Member of the acyltransferase family, has low similarity to C. elegans R02C2.3, which is a receptor protein involved in dauer larval develop
C32H11.1	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans K08D8.4
C32H11.10*	Protein containing a DUF141 domain of unknown function, has very strong similarity to uncharacterized C. elegans C32H11.9
C32H11.12	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans C32H11.9
C32H11.4	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans C32H11.3
C32H11.9*	Protein containing a DUF141 domain of unknown function, has very strong similarity to uncharacterized C. elegans C32H11.10
C35E7.1*	Protein of unknown function
C35E7.5*	Protein of unknown function
C39E9.1	Member of the SCP-like extracellular protein family, has high similarity to uncharacterized C. elegans F49E11.11
C49C3.9	C49C3.9: Protein of unknown function
C50E3.12*	Protein of unknown function
C52D10.7*	skr-9; C52D10.7 Protein with strong similarity to C. elegans SKR-8, which is involved in embryogenesis and larval development, member c
C52D10.9	skr-8; C52D10.9 Member of the Skp1-related (skr) gene family that is involved in embryogenesis and larval development, interacts with CL
C54G4.6	Member of the Maf-like protein family, which may be inhibitors of septum formation, has low similarity to uncharacterized S. pombe Spac3;
F13A7.9	skr-11; F13A7.9 Protein with high similarity to C. elegans SKR-10, which binds C. elegans CUL-1 and serves in embryogenesis and larval
F13D12.6**	Member of the serine carboxypeptidase family, has moderate similarity to cathepsin A (protective protein for beta-galactosidase, human PI
F15E11.1	F15E11.1 F15E11.1
F15E11.9*	Member of the C-type lectin family
F15E11.12	F15E11.12 F15E11.12
F22A3.6	F22A3.6: Possible lysozyme, member of an uncharacterized protein family
F23H11.7	F23H11.7: Protein of unknown function
F28B4.3	Protein containing two epidermal growth factor (EGF)-like domains, a von Willebrand factor (VWF) type A domain, and a C-type lectin dom
F28H7.3*	Member of the lipase class 3 family, which are a subset of lipolytic enzymes that hydrolyze ester linkages of triglycerides, has high similar
F35E12.5*	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans F35E12.6
F40A3.7*	sq-2: Protein of unknown function, has moderate similarity to uncharacterized C. elegans SRQ-1
F41A4.1	Protein containing three PAN domains, which may mediate protein-protein or protein-carbohydrate interactions
F45C12.7*	Protein containing a BTB (BR-C, tk and bator) or POZ (Pox virus and zinc finger) domain, which are found in some DNA and actin binding
F46E10.1	F46E10.10; F46E10.D Protein containing a lactate or malate dehydrogenase NAD binding domain and a lactate or malate dehydrogenase
F49C12.7	Protein containing a DUF227 domain of unknown function, has low similarity to uncharacterized C. elegans T16G1.4
F49E11.7	Member of the protein phosphatase protein family
F49E12.1*	Member of the animal home peroxidase family, has low similarity to eosinophil peroxidase (human EPX), which produces reactive oxidants
F49E12.2*	F49E12.2: Member of the calpain protease protein family
F49F1.1	F49F1.1 Protein of unknown function, has high similarity to uncharacterized C. elegans F49F1.6

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F52E1.1*	Member of the V-ATPase subunit H family, which are involved in vacuolar proton transport, has weak similarity to vacuolar H(+)-ATPase (F52E1.5: Protein of unknown function)
F52E1.5	F52E1.5: Protein of unknown function
F52G3.4	F52G3.4: Protein with weak similarity to C. elegans F33H12.6
F55G11.5	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans K10D11.2
F55G11.7	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans F55G11.8
F55G11.8	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans F55G11.4
F56A4.1*	Member of the astacin (M12A) family of metalloproteases, has low similarity to a region of toll-like 2 (human TLL2), which processes the
F56D6.2	Protein containing a C-type lectin domain, which mediate calcium-dependent carbohydrate recognition, has high similarity to uncharacterized
F56G4.2*	pes-2; F56G4.2: Protein of unknown function, has very strong similarity to uncharacterized C. elegans F56G4.3
F56G4.3*	F56G4.3: (pes-2) Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme.
F57F4.3	Protein of unknown function, putative paralog of C. elegans F57F4.4
F57F4.4	Protein that may be localized to a secretory vesicle or the Golgi apparatus, may be involved in body size or growth regulation
F58F6.2	Protein with high similarity to C. elegans SQT-3, which is a collagen that is involved in morphogenesis of an epithelium and cuticle synthesis
F59D8.1	F59D8.1 F59D8.1
F59D8.2*	F59D8.2: vit-4: Member of the vitellogenin protein family; expressed only in C. elegans intestinal cells
H04D03.1*	Protein of unknown function
H19N07.1*	Protein with high similarity to G1 to S phase transition 2 (mouse Gsp12), which adopt a beta barrel structure and may be a translation release
K01C8.5*	gel-14; Protein of unknown function; "GEX-3-interacting molecule"
K01G5.3*	Protein of unknown function
K02H11.2	Member of the 7-transmembrane chemoreceptor family of G protein-coupled receptors (GPCR), has low similarity to C. elegans ODR-10, 1
K06A4.1	Member of the astacin (M12A) family of metalloproteases, has a region of low similarity to a region of bone morphogenetic protein 1 (human
K06A4.5	Protein with high similarity to 3-hydroxyanthranilate 3,4-dioxygenase (human HAAO), which catalyzes the conversion of 3-hydroxyanthranilate
K08D8.5	Protein containing a DUF141 domain of unknown function, has moderate similarity to uncharacterized C. elegans C32H11.12
K10D11.1	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans F55G11.8
K12H4.7	K12H4.7: K12H4.7A Member of the carboxypeptidase protein family
M163.3*	his-24; Histone H1, 21.5 kDa form
M60.1	Protein of unknown function, has low similarity to a region of serine protease 22 (placental protein 11, human P11), which is a serine protease
R09H10.5	R09H10.5: Member of the EGF-repeat protein family
R11G1.3	Protein containing glutathione S-transferase N- and C-terminal domains, has moderate similarity to prostaglandin D2 synthase (human PG
T03E6.7	cpl-1; T03E6.7: Member of the cathepsin L-like cysteine protease protein family
T05A12.3	T05A12.3: Protein of unknown function, has weak similarity to C. elegans R07G3.3
T16A9.1**	Protein of unknown function
T18D3.4**	myo-2; mhc-2; (Myosin); T18D3.4: Protein with high similarity to sarcomeric myosin heavy chain (C. elegans UNC-54), which is a component
T24B8.5*	T24B8.5: Protein of unknown function, has weak similarity to C. elegans F49F1.7
T25C12.3	T25C12.3: Member of the EGF-repeat protein family, member of the C-type lectin family
W01A11.4	Member of the galectin family, which are lectins that bind beta galactosides, has low similarity to a region of galectin 8 (rat Lgalectin 8), which is
W02D9.7	W02D9.7: Protein of unknown function
Y106G6H.10	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme
Y106G6H.9	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme
Y14H12B.2*	Protein of unknown function, has moderate similarity to uncharacterized C. elegans C16A11.4
Y19D10A.9*	Protein containing a C-type lectin domain, which mediate calcium-dependent carbohydrate recognition, has very strong similarity to uncharacterized
Y22F5A.5	Member of an uncharacterized protein family with weak similarity to Entamoeba histolytica N-acetylmuramidase
Y37D8A.12**	Protein of unknown function, has very strong similarity to uncharacterized C. elegans W03G9.3
Y38E10A.14*	Y38E10A.14 Y38E10A.14
Y38H6C.1	Protein of unknown function, has high similarity to uncharacterized C. elegans M02H5.8
Y38H6C.3*	Protein of unknown function, has moderate similarity to uncharacterized C. elegans K07C11.10
Y38H6C.5	Member of the retrotransposon gag protein family, contains a zinc knuckle domain, which can bind RNA or DNA in eukaryotes
Y39B6B.gg*	Y39B6A.1: Protein with weak similarity to flaggrin (human FLG), which is a rheumatoid arthritis autoantigen that may have varied roles related
Y43F4A.3*	Protein of unknown function
Y45F10A.2*	Protein containing eight Pumilio-family (Puf) RNA binding domains, has high similarity to a region of C. elegans FBF-1, which is an RNA-binding
Y46C8_103.a	Y46C8_103.a Y46C8_103.a
Y46H3C_14.c	Y46H3C_14.c Y46H3C_14.c
Y49E10.1*	rpt-6; Putative ATPase subunit of 19S proteasome particle that functions in embryonic and larval development

Y49E10.8*	Y49E10.8: Protein of unknown function
Y51H7BR.2	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme, has high similarity
Y55B1AR.1	Y55B1AR.1: Protein with weak similarity to C. elegans W06H1.6 (Galactoside-binding lectin) Protein that binds sugars
Y56A3A.15	Protein containing an F-box domain, which serve as a link between a target protein and a ubiquitin-conjugating enzyme, has strong similar
Y62H9A.3*	Y62H9A.3: Protein of unknown function, has weak similarity to C. elegans Y62H9A.5
Y62H9A.4*	Y62H9A.4: Protein of unknown function, has weak similarity to C. elegans Y62H9A.6
Y62H9A.5	Y62H9A.5: Protein of unknown function, has weak similarity to C. elegans Y62H9A.3
Y62H9A.6*	Y62H9A.6: Protein of unknown function, has weak similarity to C. elegans Y62H9A.4
Y9D1A.1*	Y9D1A.1: Protein with moderate similarity to C. elegans Y9D1AA
ZK1127.3*	Protein of unknown function
ZK1127.10	Protein with high similarity to cystathionase (human CTH), which is a pyridoxal phosphate-dependent enzyme that acts in the conversion o
ZK1251.2	Ins-7; ZK1251.2 Insulin-like protein of the type-beta subfamily
ZK6.10	Member of the protein of unknown function (DUF274) family, has high similarity to uncharacterized C. elegans ZK6.11
ZK6.11*	Member of the protein of unknown function (DUF274) family, has high similarity to uncharacterized C. elegans ZK6.10
ZK757.1	Protein containing a DHHC-type zinc finger (NEWH) domain, has a region of low similarity to a region of huntingtin interacting protein H (ht
ZK896.5	Protein containing a DUF141 domain of unknown function, has high similarity to uncharacterized C. elegans ZK896.4
ZK896.7**	Putative paralog of C. elegans Y73C8C.2, has similarity at the N-terminus to C. elegans F38A1.5, a member of the phospholipase A2 rece
ZK896.8*	gcy-18; gcy-26; ZK896.8 Protein containing a receptor family ligand binding domain, a protein kinase domain and an adenylate and guanyl

\*: Genes that also scored significantly in SAM analysis

\*\* : Genes with lower fold-difference but consistently downregulated (identified through SAM analysis)

In the set of mutant arrays, 60 upregulated and 54 downregulated genes were found to be significant;

q-value = 0.0011197 with 0.6207 median false significant genes, at delta = 1.58.

Annotations are from WormPD ([www.incyte.com/proteome/WormPD](http://www.incyte.com/proteome/WormPD))

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## 2s: Lifespan data

Mantel-Cox log-rank method was used to determine p-values.

(a) *fer-15; daf-2(mu150); fem-1*, 25°C

RNAi	Gene/description	mean	std err	p-value	% vector
C02A12.4	<i>lys-7</i> N-acetylmurami	18.3	0.92	<0.0001	72.6
	<i>daf-16</i>	11.5	0.42	<0.0001	45.5
	<i>daf-2</i>	13.9	0.66	<0.0001	55.1
F28D1.3	thumatin	18.4	0.93	<0.0001	73.0
T10B9.1	Cyt P450	15.4	0.81	<0.0001	61.0
Y54G11A.5b	<i>ctf-2</i>	13.6	0.76	<0.0001	54.0
T20G5.7	unknown	18.6	0.94	0.0008	79.8
F38E11.2	<i>hsp-12.6</i>	19.1	0.94	0.0015	75.8
B0213.15	Cyt P450	30	0.83	0.0025	119.0
K11G9.6	<i>mtl-1</i>	19.1	1.1	0.0025	75.8
C05E4.9	<i>gel-7</i> malate synthas	19.4	1.05	0.0027	77.1
F10D2.9	<i>fat-7</i>	20.1	1.1	0.0269	79.8
C24B9.4	unknown	19.8	1.06	0.031	78.5
control		25.2	1.3		

(b) *daf-2(mu150)*, 20°C-25°C shift at late L3

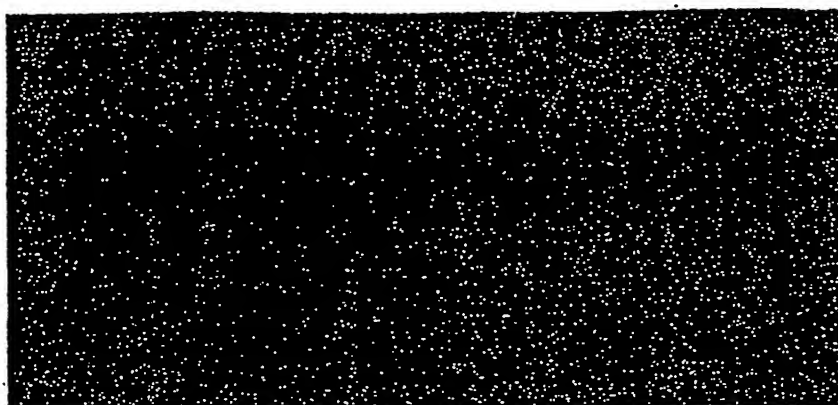
RNAi	Gene/description	mean	std err	p-value	% vector
Y54G11A.6	<i>ctf-1</i>	33.6	1.07	<0.0001	87.8
K07C6.4	Cyt P450	34.7	0.88	<0.0001	90.6
	<i>daf-16</i>	16.6	0.80	<0.0001	43.3
T28B8.2	<i>ins-18</i>	36.0	0.84	0.0002	94.1
Y54G11A.5b	<i>ctf-2</i>	35.6	0.88	0.0007	92.9
	<i>daf-2</i>	40.6	1.15	0.0009	106.1
C08A9.1	<i>sod-3</i>	35.5	1.03	0.0163	92.6
K07E3.3	<i>dao-3</i> THF synthetas	35.8	1.05	0.0196	93.4
control		38.3	1.12		

(c) *daf-2(mu150)*, 20°C -25°C shift at L3

RNAi	Gene/description	mean	std err	p-value	% vector
C50B8.2	<i>bir-2</i> apoptosis	23.3	1.00	<0.0001	81.1
Y54G11A.6	<i>ctf-1</i>	23.7	0.86	<0.0001	82.6
	<i>ctf-1+ctf-2</i>	24.7	0.86	<0.0001	86.1
	<i>daf-16</i>	15.1	0.34	<0.0001	52.5
	<i>daf-2</i>	31.2	0.70	<0.0001	108.4
T10B9.1	Cyt P450	19.8	0.64	<0.0001	68.8
K11D2.2	ASAH acid ceramidas	24.6	0.90	0.0013	85.4
K07C6.4	Cyt P450	24.4	0.87	0.0015	84.9
F38E11.2	<i>hsp-12.6</i>	25.7	0.90	0.0019	89.4
C52E4.1	<i>gcp-1</i> gut cysteine pr	25.6	0.83	0.0031	89.2
C46F4.2	acyl-CoA synthetase	25.2	0.88	0.0035	87.8
K07E3.3	<i>dao-3</i> THF synthetas	24.1	1.01	0.0038	83.9
C06B3.4	estradiol 17b dh	25.2	0.83	0.0088	87.6
T28B8.2	<i>ins-18</i>	25.3	0.84	0.0133	88.1
K11G9.6	<i>mtl-1</i>	25.7	0.89	0.0151	89.4
F11A5.12	estradiol 17b dh	25.5	0.83	0.0158	88.6
F43D9.4	<i>slp-1 hsp</i>	25.4	0.92	0.0203	88.4
B0213.15	Cyt P450	30.6	0.57	0.0206	106.4
K12G11.4	alcohol dh	25.9	0.89	0.0384	90.1
Y54G11A.5b	<i>ctf-2</i>	25.8	0.92	0.0439	89.6
K12G11.3+.4		25.6	0.80	0.047	89.1

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control 28.8 0.74

(d) *daf-2(mu150)*, 20°-25°C shift at L2

RNAi	Gene/description	mean	std err	p-value	% vector
T22G5.7	saposin type B	22.4	1.43	0.0001	79.2
F32A5.5	aquaporin	22.3	1.62	0.0001	78.7
<i>ctl-1</i> +K07C6.4		20.5	1.24	0.0001	72.4
	<i>daf-16</i>	14.9	0.72	0.0001	52.5
T20G5.7	meditritin-like ShK toxir	23.3	1.53	0.0002	82.3
T27E4.8	<i>hsp-16.1</i>	20.2	1.01	0.0002	71.3
C02A12.4	<i>lys-7</i> lysozyme	22.6	1.49	0.0003	79.8
T27E4.2	<i>hsp-16.11</i>	23.0	1.50	0.0007	81.4
T27E4.9	<i>hsp-16.49</i>	22.9	1.43	0.0009	81.0
T20G5.8	meditritin-like ShK toxir	23.9	1.56	0.0022	84.3
Y48H3A.3	<i>hsp-16.2</i>	25.6	1.41	0.0084	90.4
T07C4.4	<i>spp-1</i> saposin	23.9	1.57	0.0089	84.3
R03E9.1	<i>mdl-1</i>	25.8	1.62	0.0309	91.1
control		28.3	1.58		100.0

(e) *daf-2(e1370)*, 20-25.5 shift at L4: Antibacterials

RNAi	Gene/description	mean	std err	p-value	% vector
C02A12.4	<i>lys-7</i> lysozyme	13.7	1.01	0.0001	56.1
T20G5.8	meditritin-like ShK toxir	17.1	0.83	0.0011	70.0
T07C4.4	<i>spp-1</i> saposin	18.9	1.27	0.0242	77.0
T22G5.7	saposin type B	19.5	1.24	0.0485	79.7
control		24.5	1.55		100.0

(f) *fer-15; fem-1*, 25°C: Group 2 RNAs from (j)

RNAi	Gene/description	mean	std err	p-value	% vector
C54G4.6	MAF/ASMTL	16.9	0.76	<0.0001	129.2
	<i>daf-2</i>	17.1	0.83	0.0002	130.5
C04F6.1	<i>vit-5</i>	15.9	0.63	0.0008	121.5
control		13.1	0.53		

(g) *rrf-3(pk1426)*, 20°C

RNAi	Gene/description	mean	std err	p-value	% vector
	<i>daf-2</i>	31.1	0.75	<0.0001	207.3
ZK1251.2	<i>ins-7</i>	23.3	0.51	<0.0001	155.2
C32H11.12	unknown	19.7	0.68	0.0001	131.3
C54G4.6	ASMTL/MAF	19.9	0.66	0.0004	132.4
ZK896.8	<i>gcy-18</i>	18.8	0.73	0.0058	125.5
C42D8.2	<i>vit-2</i>	18.2	0.58	0.0165	121.0

control 15.0 0.61

(h) *rrf-3(pk1426)*, 20°C through late L2, shifted to 25°C, back to 20 as Day 1 adults.

RNAi	Gene/description	mean	std err	p-value	% vector
C54G4.6	ASMTL/MAF	19.0	0.58	<0.0001	126.4
C32H11.12	unknown	18.7	0.72	<0.0001	124.4
	<i>daf-16</i>	11.9	0.37	<0.0001	79.3
	<i>daf-2</i>	28.7	1.57	<0.0001	191.5
B0024.6	<i>gcy-6</i>	18.9	0.51	<0.0001	126.1
ZK1251.2	<i>ins-7</i>	20.0	1.01	<0.0001	133.3
K10D11.1	unknown	20.1	0.68	<0.0001	133.7
C07B5.1	<i>nuc-1</i> endonuclease	19.6	0.56	<0.0001	130.4
ZK6.10	unknown	19.2	0.80	<0.0001	127.9
ZK896.8	<i>gcy-18</i>	18.7	0.72	0.0001	124.7
B0554.6	unknown	18.5	0.64	0.0002	123.0
C42D8.2	<i>vit-2</i>	18.7	0.69	0.0002	124.4
C32H11.10	unknown	18.3	0.70	0.0004	121.7
F13B12.5	<i>ins-1</i>	18.3	0.70	0.0004	121.9
T08G5.10	<i>mtl-2</i>	18.0	0.60	0.0004	120.2
F55G11.5	unknown	17.7	0.76	0.0019	118.1
F49E12.2	calpain protease	17.5	0.78	0.0048	116.5
C04F6.1	<i>vit-5</i>	16.5	0.51	0.0056	109.7
T22G5.2	<i>lbp-7</i> fatty-acid bindir	17.2	0.57	0.0132	114.4
K04E7.2	<i>pep-2</i> oligopeptide tr	17.1	0.61	0.0165	113.7
C08H9.5	<i>old-1</i> tyr. kinase	16.7	0.83	0.0234	111.6

control 15.0 0.61

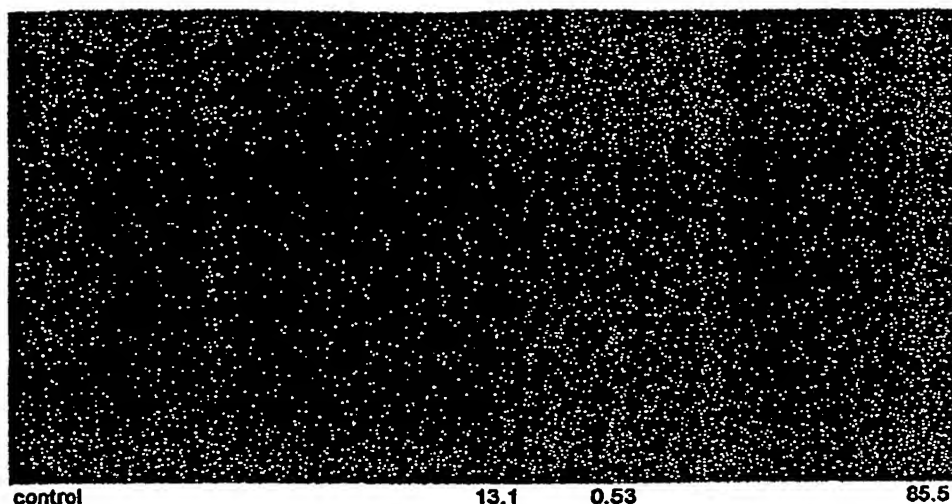
(i) *rrf-3(pk1426)*, 20°C until L2/L3, shifted to 25°C, back to 20°C at Day 1

RNAi	Gene/description	mean	std err	p-value	% vector
F56G4.2	<i>pes-2</i>	20.0	0.99	0.0053	124.5

control 16.1 0.80 100.0

(j) *fer-15; fem-1*, 25°C

RNAi	Gene/description	mean	std err	p-value	% vector	% avg. V
C54G4.6	ASMTL/MAF	16.9	0.76	<0.0001	129.2	110.4
	<i>daf-2</i>	17.1	0.83	0.0002	130.5	111.5
C04F6.1	<i>vit-5</i>	15.9	0.63	0.0008	121.5	103.8
ZK1320.2	unknown	14.6	0.46	0.0017	111.7	95.4
ZK270.2	unknown	10.5	0.54	0.0018	80.5	68.8
T10B9.1	Cyt. P450	10.9	0.44	0.0035	83.2	71.1
K07A1.7	unknown	15.1	0.62	0.0062	115.4	98.6
ZK355.E	unknown	15.2	0.59	0.0083	116.0	99.2
H22K11.1	<i>asp-3</i>	15.0	0.71	0.0135	114.5	97.8
C17G1.4	mucin	11.4	0.48	0.0181	86.9	74.2



control 13.1 0.53 85.5

(k) *fer-15; fem-1*, 25°C

RNAi	Gene/description	mean	std err	p-value	% vector	% avg. V
B0554.1	unknown	12.7	0.42	<0.0001	86.8	82.6
B0554.6	unknown	12.4	0.40	<0.0001	85.1	81.1
C17G1.4	mucin	12.2	0.41	<0.0001	83.6	79.6
C32H11.10	unknown	12.9	0.49	<0.0001	88.5	84.3
C32H11.12	unknown	12.1	0.38	<0.0001	82.9	78.9
C55B7.4	acyl-CoA dh	13.0	0.43	<0.0001	89.3	85.1
	<i>daf-16</i>	8.3	0.29	<0.0001	57.1	54.4
F28D1.3	thaumatin	12.3	0.44	<0.0001	84.1	80.1
F28D1.5	thaumatin	12.3	0.46	<0.0001	84.2	80.2
F55G11.5	unknown	12.1	0.36	<0.0001	82.9	78.9
K04E7.2	<i>pep-2</i>	10.6	0.32	<0.0001	72.6	69.1
K07C6.4	Cyt.P450	12.1	0.41	<0.0001	83.1	79.1
T10B9.1	Cyt.P450	10.8	0.31	<0.0001	73.8	70.3
W08D2.4	<i>fat-3</i> f.a. desaturase	12.1	0.24	<0.0001	83.1	79.1
ZK384.4	unknown	12.6	0.40	<0.0001	86.3	82.2
ZK1251.2	<i>ins-7</i>	16.4	0.19	0.0001	112.3	107.0
C46F4.2	acyl-CoA synthetase	13.3	0.39	0.0002	91.3	87.0
K12G11.3	alcohol dh	12.6	0.40	0.0002	86.2	82.1
W06D12.3	<i>fat-5</i> palmitoyl-CoA d	13.7	0.43	0.0005	93.8	89.3
T13F2.1	<i>msp</i>	13.1	0.37	0.0008	89.9	85.6
C32F10.4	<i>lin-35</i>	13.6	0.38	0.0014	93.1	88.6
Y38H6C.5	Zn finger	13.3	0.37	0.0036	90.8	86.4
B0213.15	Cyt.P450	14.4	0.37	0.0129	88.3	93.6
F11A5.12	est. 17 b dh	13.9	0.41	0.0147	95.4	90.9
C24B9.9	unknown	14.1	0.38	0.0165	96.7	92.1
C54D10.1	<i>gst</i> glutathione S-tran	13.6	0.37	0.0246	92.9	88.5

control		14.6	0.37			95.2
(l) <i>fer-15; fem-1</i> , 25°C						
RNAi	Gene/description	mean	std err	p-value	% vector	% avg. V
	<i>daf-16</i>	11.3	0.28	<0.0001	66.5	73.7
C54G4.6	ASMTL/MAF	18.7	0.64	0.0021	110.1	122.0
control		17.1	0.57			111.3

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